BMP2-mediated alteration in the developmental pathway of fetal mouse brain cells from neurogenesis to astrocytogenesis

Kinichi Nakashima*†‡, Takumi Takizawa*†‡§, Wataru Ochiai*†, Makoto Yanagisawa*†, Tatsuhiro Hisatsune¶, Masato Nakafuku‖, Kohei Miyazono††, Tadamitsu Kishimoto‡‡, Ryoichiro Kageyama§§, and Tetsuya Taga*†¶¶**

*Department of Cell Fate Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan; †Department of Molecular Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 101-0062, Japan; §Department of Pediatrics, School of Medicine, Gunma University, Gunma 371-8511, Japan; ¶Division of Integrated Biosciences, Graduate School of Frontier Science, University of Tokyo, Tokyo 113-8657, Japan; ‖Division of Neurobiology, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan; **Department of Biochemistry, The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo 170-8455, Japan; ††Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan; ‡‡Osaka University, Osaka 565-0871, Japan; and §§Department of Cell Biology, Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan

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We show that when telencephalic neural progenitors are briefly exposed to bone morphogenetic protein 2 (BMP2) in culture, their developmental fate is changed from neuronal cells to astrocytic cells. BMP2 significantly reduced the number of cells expressing microtubule-associated protein 2, a neuronal marker, and cells expressing nestin, a marker for undifferentiated neural precursors, but BMP2 increased the number of cells expressing S100-β, an astrocytic marker. In telencephalic neuroepithelial cells, BMP2 up-regulated the expression of negative helix–loop–helix (HLH) factors Id1, Id3, and Hes-5 (where Hes is homologue of hairy and Enhancer of Split) that inhibited the transcriptional activity of neurogenic HLH transcription factors Mash1 and neurogenin. Ectopic expression of either Id1 or Id3 (where Id is inhibitor of differentiation) inhibited neurogenesis of neuroepithelial cells, suggesting an important role for these HLH proteins in the BMP2-mediated changes in the neurogenic fate of these cells. Because gliogenesis in the brain and spinal cord, derived from implanted neural stem cells or induced by injury, is responsible for much of the failure of neuronal regeneration, this work may lead to a therapeutic strategy to minimize this problem.

Precise mechanisms by which neurogenesis and gliogenesis are regulated in the central nervous system (CNS) remain to be elucidated. Fetal telencephalic neuroepithelial cells contain neural precursors that give rise to the neuronal lineage and the glial lineage, which includes astrocytes and oligodendrocytes (1, 2). The fate of neural precursors in the developing brain is believed to be determined by intrinsic cellular programs and by external cues, including the cytokines (1, 2). Bone morphogenetic protein 2 (BMP2) is a pleiotropic cytokine (3) that is active in many tissues including the CNS (for review, see ref. 4). The action of BMP2 is mediated by heterotetrameric serine/threonine kinase receptors and the downstream transcription factors Smad1, -5, or -8. After these transcription factors are phosphorylated on serines, they form a complex with a common mediator, Smad4, and the complex is translocated into the nucleus to activate transcription of specific genes (5–7). Inhibitory Smad proteins, Smad6 and Smad7, repress the action of BMP2 by inhibiting the receptor-mediated phosphorylation of Smad1, -5, or -8 or by competing with Smad4 for the binding to Smad1, -5, and -8 (5–10). BMP2 can promote telencephalic neuroepithelial cells to differentiate as astrocyte (11, 12). We have further demonstrated (12, 13) that BMP2 and leukemia inhibitory factor (LIF) act synergistically to induce neuroepithelial cells to become astrocytes by forming a complex of the respective downstream transcription factors, Smads and STAT3, bridged by p300. In the present study, we demonstrate that BMP2 not only promotes astrocyte differentiation in cooperation with LIF but also alters the neurogenic cell fate of telencephalic progenitors so that they develop into astrocytic cells. We found that a brief exposure of neuroepithelial cells to BMP2 is sufficient to irreversibly change their differentiation program and to prepare them to undergo astrocytogenesis, after the addition of LIF. It has also been suggested that BMP2 has an antineurogenic effect (4, 11, 14–16), but the molecular mechanism remains largely elusive. In this paper, we show that the presence of BMP2 alters the differentiation of proliferating neural precursor cells from a neuronal to an astrocytic fate, and we propose a mechanism by which BMP2 signaling interacts with neurogenic transcription factors to induce this effect.

Generally, the determination of cell fate involves transcription factors. In the nervous system, for instance, neurogenesis is promoted by proneural basic helix–loop–helix (bHLH) transcription factors such as Mash1 (mammalian achaete-scute homologue), neurogenin, and NeuroD. These tissue-specific bHLH factors form heterodimers with ubiquitously expressed bHLH proteins such as *E2A* gene products, E12 and E47 (17–19), and thus activate the transcription of genes that have a CANNTG sequence (E box) in the promoter region (17–19). Functions of the bHLH proteins are negatively regulated by another set of HLH factors, Hes-1 and Hes-5 (where Hes is homologue of hairy and Enhancer of Split). These factors are mammalian bHLH proteins that are distant relatives of the product of *Drosophila* pair-rule gene hairy (20–22), they are induced by Notch signaling, and they inhibit Mash1 function by competitively binding to its heterodimeric bHLH partners, E12 and E47 (20–22). Another type of negative regulator for bHLH proteins is the Id (inhibitor of differentiation) family of proteins, which have an HLH domain but lack a basic region (23–25). Id proteins inhibit the function of myogenic bHLH proteins, such as MyoD, by a mechanism analogous to that used by Hes proteins (26). We show herein that negative HLH proteins contribute to the antineurogenic effect of BMP2. It should also be emphasized that the target gene *Hes-5*, downstream of Notch, is unexpectedly induced by BMP2, suggesting existence of cross-talk between Notch and BMP signaling.

Abbreviations: BMP, bone morphogenetic protein; MAP2, microtubule-associated protein 2; HLH, helix–loop–helix; Hes, homologue of hairy and Enhancer of Split; Id, inhibitor of differentiation; LIF, leukemia inhibitory factor; CNS, central nervous system; BrdUrd, bromodeoxyuridine.

‡K.N. and T. Takizawa contributed equally to this work.

¶¶To whom reprint requests should be addressed at: Department of Cell Fate Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1, Honjo, Kumamoto 860-0811, Japan. E-mail: taga@kaiju.medic.kumamoto-u.ac.jp.

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In this report, we demonstrate the irreversible alteration of CNS neural precursor cells from neurogenesis to astrocytogenesis mediated by BMP2 via a mechanism involving negative HLH proteins. We also discuss possible clinical applications of our findings to minimize the undesirable gliogenesis that occurs after neural stem cell implantation and nerve injury.

Methods

Cell Culture. Neuroepithelial cells were prepared from embryonic day 14 mouse telencephalons as described (12, 13). Freshly isolated cells were allowed to proliferate for 4 days in dishes precoated with poly(L-ornithine) and fibronectin containing N2-supplemented DMEM/F-12 and basic fibroblast growth factor (bFGF) at 10 ng/ml. bFGF was included unless otherwise indicated. Cells were then detached and replated on chamber slides precoated with poly(L-ornithine) and fibronectin (Nunc; 1×10^5 cells per well) or 12-well plates (Nunc; 8×10^5 cells per well) for immunofluorescent and luciferase assays, respectively. Animals were treated according to the guidelines of the Tokyo Medical and Dental University Animal Committee.

Immunocytochemistry. Cells cultured on chamber slides were fixed with 4% paraformaldehyde in PBS and subjected to immunofluorescent staining. The following primary antibodies were used: Mouse mAbs were anti-MAP2 (microtubule-associated protein 2; diluted 1:500; Sigma), anti-S100-β (diluted 1:250; Sigma), biotin-conjugated anti-bromodeoxyuridine (BrdUrd; diluted 1:20; ALEXIS, San Diego), biotin-conjugated anti-FLAG (1:100; Sigma), and anti-nestin (1:100; PharMingen). Rabbit polyclonal antibodies were anti-nestin (diluted 1:300; provided by K. Yoshikawa, Osaka Univ.), anti-glial fibrillary acidic protein (GFAP; diluted 1:2000; Dako), and anti-LacZ (diluted 1:1000; Molecular Probes). The following secondary antibodies were used: FITC-conjugated goat anti-mouse IgG antibody (diluted 1:100; Jackson ImmunoResearch), streptavidin-conjugated Texas Red (diluted 1:500; Vector), and rhodamine-conjugated donkey anti-rabbit IgG antibody (diluted 1:100; Chemicon, Temecula, CA). The cells were counterstained with Hoechst 33258 to identify nuclei. Images were obtained with fluorescent microscopy on an AX70 microscope (Olympus, New Hyde Park, NY).

Luciferase Assay. Neuroepithelial cells were transfected with a luciferase reporter gene fused to a fragment of *Hes-5* promoter (nucleotides −179 to +72; −179H5-Luc) or its 5′ terminally truncated versions (−141H5-Luc or −66H5-Luc) by using *Trans*-IT LT-1 (Mirus) according to the manufacturer's protocol. Luciferase reporter constructs with a 1.6-kb fragment (nucleotides −1575 to +88) of *Id1* promoter (−1575Id1-Luc) or its 5′ terminally truncated versions (−1147Id1-Luc or −927Id1-Luc) were also used. A seven-E-box (CAGGTG)-containing luciferase reporter construct (pKE7-βA(core)-luc) was also used. As an internal control, a plasmid containing sea pansy luciferase expression construct (pRL-CMV; Promega) was co-transfected with the reporter constructs described above. The following expression constructs were also used: FLAG-tagged *Smad7* in pcDEF3; *Mash1* and *E47* in pSV2CMV; and FLAG-tagged *Hes-5*, *Id1*, and *Id3* in pEF-BOS. On the following day, some cells were treated with BMP2 at 80 ng/ml for 8 h and other cultures were not treated. After the 8-h incubation, cells were solubilized and luciferase activity was measured according to as recommended for the Pikkagene dual luciferase assay system (Toyo Ink, Tokyo). Luminous CT-9000D (Dia-Iatron, Tokyo) was used for quantitation.

Recombinant Adenovirus Construction and Infection. Recombinant adenoviruses were constructed as described (27). Infection of neuroepithelial cells with each recombinant adenovirus was done at a multiplicity of infection of 200 plaque-forming units per cell.

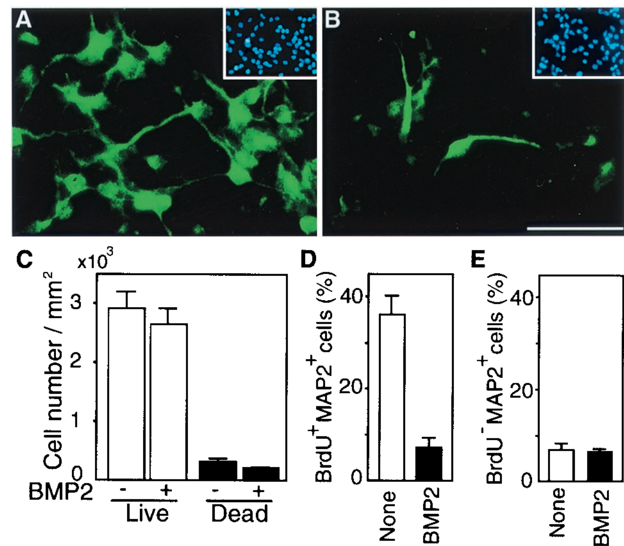

Fig. 1. Antineurogenic effect of BMP2. Neuroepithelial cells were cultured for 2 days with medium alone (*A*) or BMP2 at 80 ng/ml (*B*) and then subjected to immunofluorescent staining for MAP2 (green). (Bar, 50 μm.) (*A* and *B Insets*) Hoechst staining of the same fields (blue). (*C*) Living and dead cells were counted after a 2-day culture with or without BMP2 at 80 ng/ml. (*D* and *E*) Cells were grown in the presence of BrdUrd (200 μM) for 2 days with or without BMP2 (80 ng/ml). Percentage of cells that contained BrdUrd and/or MAP2 was determined.

Results

BMP2-Induced Suppression of Neurogenesis. As shown in Fig. 1, BMP2 dramatically reduced the number of neurons in a 2-day culture of neuroepithelial cells. The percentage of total live cells with the neuronal marker MAP2 on day 2, in the control and BMP2-treated cultures, respectively, was 42.8% and 13.6%. Only a 4-h exposure of neuroepithelial cells to BMP2 was sufficient to cause dramatic reduction in the number of MAP2-positive cells 2 days later, indicating that the antineurogenic effect of BMP2 was irreversible (data not shown). Hoechst nuclear staining suggested that cell death was apparently not induced by BMP2 (Fig. 1 *A Inset* and *B Inset*). In fact, BMP2 did not significantly affect the number of living or dead cells after 2 days (Fig. 1*C*), indicating that the reduction in the number of MAP2-positive cells by BMP2 was not caused by the specific killing of neurons but may be caused by inhibiting proliferating neural precursor cells from developing into neurons. To examine this hypothesis, the thymidine analog BrdUrd was included in neuroepithelial cell cultures throughout the 2-day culture period. Among total living cells, the percentage of BrdUrd-labeled cells in BMP2-treated cultures (84.4%) and untreated cultures (89.2%) was similar. Nevertheless, cells positive for both BrdUrd and MAP2 were markedly reduced in BMP2-treated cultures (Fig. 1*D*). In contrast, the number of BrdUrd-unlabeled neurons that contained MAP2, considered to be postmitotic neurons and present from the beginning of the 2-day culture, was similar in cultures with or without BMP2 (Fig. 1*E*). Thus, BMP2 appears to inhibit the neurogenic fate of proliferating precursors.

BMP2-Induced Switch in the Fate of Neural Precursor Cells. To determine whether BMP2 maintained neural precursor cells in an undifferentiated state or altered their cell fate from neurogenesis to other lineage specification, we examined expression of nestin, a marker for undifferentiated neural precursor cells (28).

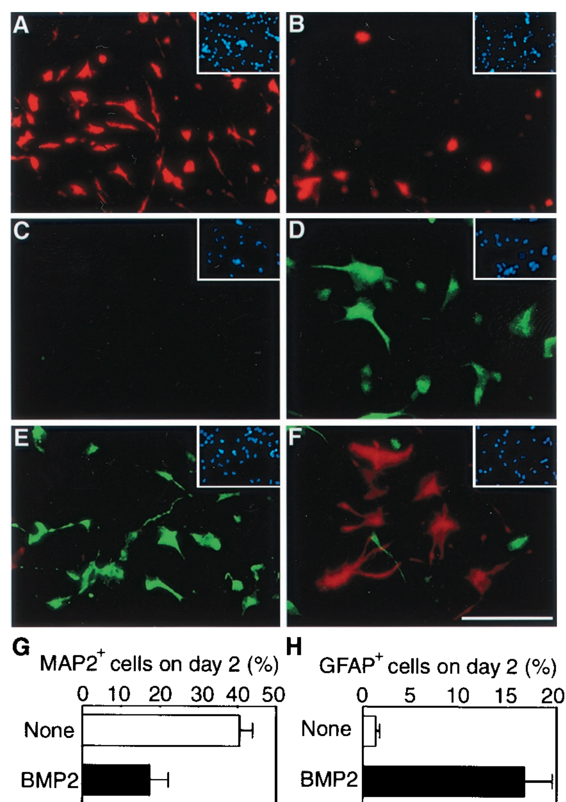

Fig. 2. BMP2-induced neurogliogenic fate conversion of neural precursors. (*A* and *B*) Nestin expression (red) in neuroepithelial cells cultured with (*B*) or without (*A*) BMP2 (80 ng/ml) for 2 days was examined. (*C* and *D*) S100-β expression (green) was examined in neuroepithelial cells cultured with (*D*) or without (*C*) BMP2 (80 ng/ml) for 2 days. (*E* and *F*) Neuroepithelial cells were stimulated (*F*) or unstimulated (*E*) with BMP2 for 4 h and washed twice with N2-supplemented DMEM/F-12. Cells were then cultured for 2 days in the absence of bFGF and subjected to immunofluorescent staining for MAP2 (green) and GFAP (red). (*G* and *H*) Neuroepithelial cells were treated and stained as in *E* and *F*. The percentage of total cells that were MAP2-positive (*G*) and GFAP-positive (*H*) was calculated. (Bar, 50 μm.) (*Insets*) Hoechst staining of the same fields (blue).

BMP2 dramatically decreased the number of nestin-positive cells (Fig. 2*B*; 9.3% of the live cells were nestin-positive) compared with that in untreated cultures (Fig. 2*A*; 35.5%), suggesting that BMP2 did not keep the precursors in an undifferentiated state but rather promoted differentiation into a nonneuronal lineage (or lineages). Because we have demonstrated (12, 13) that BMP2 and LIF synergistically induce fetal telencephalic neuroepithelial cells to differentiate into astrocytes, we hypothesized that BMP2 may have the potential to switch the fate of neural precursor cells from a neuronal to an astrocytic lineage. In support of this hypothesis, the number of cells expressing S100-β, an astrocytic cell marker, was increased after 2 days of BMP2 stimulation (Fig. 2 *C* and *D*). However, in consistent with our previous results (12, 13), no cells immunoreactive to antibody against GFAP, another astrocytic cell marker, were detected after 2 days of culture with BMP2 (data not shown). It has been reported (2, 29, 30) that proliferation of neural precursors in the *in vitro* cultures is fully dependent on the presence of a mitogen, such as bFGF or epidermal growth factor, and withdrawal of the mitogen leads to growth arrest and initiation of spontaneous differentiation of the neural precursor cells into, for instance, neurons and astrocytes. To examine whether BMP2 pulse-stimulation affects this spontaneous differentiation under the condition where no cell growth occurs, we treated neuroepithelial cells with BMP2 for 4 h and then cultured them without bFGF for additional 2 days. The number of live cells in the beginning and at the end of the culture period was similar (1,651 $\pm$ 156 cells per mm^2 on day 0, 1,618 $\pm$ 117 cells per mm^2 on day 2 without BMP2 pretreatment, and 1,740 $\pm$ 360 cells per mm^2 on day 2 with BMP2 pretreatment; mean $\pm$ SD). However, the percentage of MAP2-positive cells was greatly decreased in response to the BMP2 pulse-stimulation, and conversely the percentage of GFAP-positive cells was dramatically increased by BMP2 (Fig. 2 *E*–*H*). Thus, BMP2 appears to change the fate of neural precursors from neuronal to astrocytic cells.

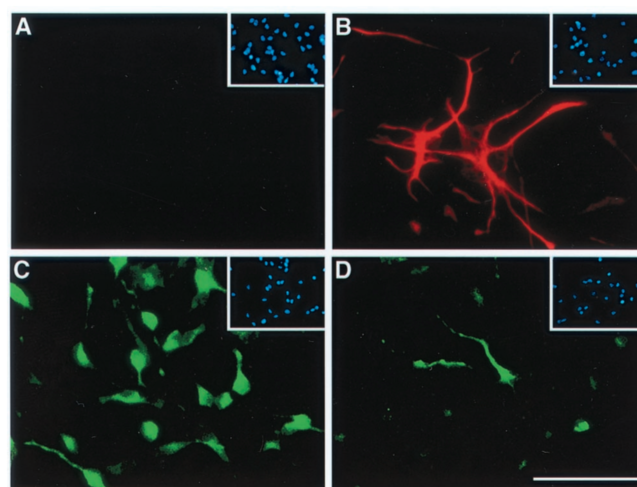

Fig. 3. Fate-converting and differentiation-promoting effect of BMP2 and LIF, respectively. (*A* and *B*) Neuroepithelial cells were treated with BMP2 for 4 h and then washed twice with N2-supplemented DMEM/F-12. The cells were then cultured for 2 days in the absence (*A*) or presence (*B*) of LIF and subjected to immunofluorescent staining for GFAP (red). (*C* and *D*) MAP2 immunostaining (green) was performed on gp130-deficient cells cultured with (*D*) or without (*C*) BMP2 (80 ng/ml) for 2 days. (Bar, 50 μm.) (*Insets*) Hoechst staining of each field (blue).

Fate-Converting and Differentiation-Promoting Effect of BMP2 and LIF, Respectively, on Neural Precursor Cells. BMP-2 stimulation is not sufficient to induce the complete astrocyte differentiation that accompanies expression of GFAP in bFGF-containing cultures (12, 13). As shown in Fig. 3*B*, a 4-h pulse-stimulation with BMP2 and subsequent 2-day culture with LIF led to the appearance of a significant number of stellate-shaped cells expressing GFAP, suggesting that BMP2 inhibited the neurogenic fate of neural precursor cells and enabled them to differentiate into astrocytes in the presence of LIF. In fact, when LIF was removed from the culture after a 4-h exposure to BMP2, no GFAP-positive stellate-shaped cells were detected (Fig. 3*A*). Telencephalic neuroepithelial cells spontaneously express LIF and related cytokines [i.e., IL-6 family cytokines sharing gp130 as a receptor component critical for signal transduction (31)], as well as their receptor components (12, 13). However, a possible cooperative involvement of such endogenously expressed gp130-stimulating cytokines in the antineurogenic effect of BMP2 was clearly excluded because neuronal differentiation was effectively suppressed by BMP2, even in the absence of gp130 signaling (Fig. 3 *C* and *D*).

Smad-Dependent Induction of Negative HLH Regulators. We next investigated the mechanism of BMP2-induced suppression of neurogenesis in neural precursor cells. Because one of the tissue-specific class of bHLH proteins, Mash1, plays an essential role in neuronal differentiation (17–19), we examined whether

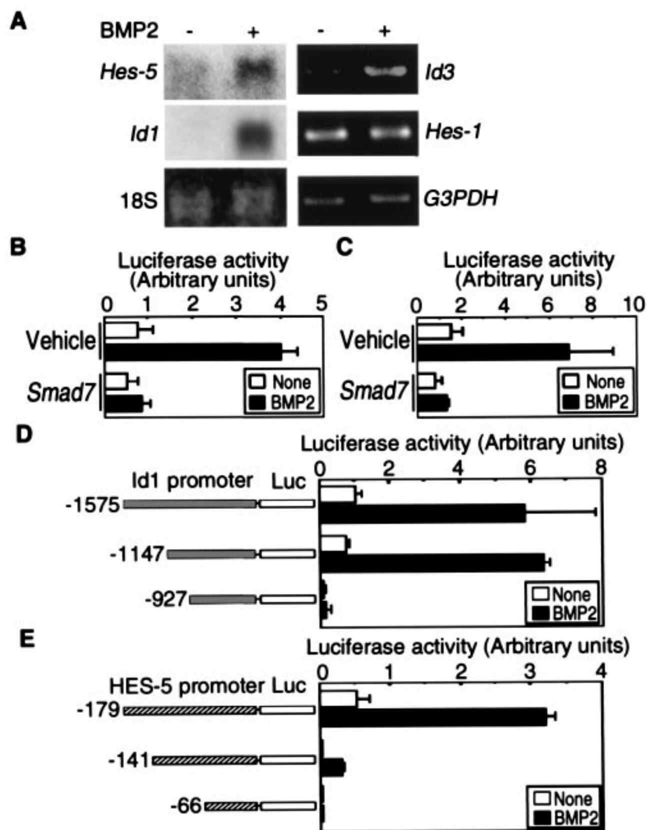

Fig. 4. Smad-dependent induction of negative HLH regulators. (*A*) Total RNAs were extracted from cells treated or untreated with BMP2 (80 ng/ml) for 1 h and then subjected to Northern blot analysis with a specific probe for *Hes-5* or *Id1* (*Left*). Ethidium bromide staining showing ribosomal RNA (18S) was also indicated as a control. Total RNAs from the same sources were analyzed by reverse transcriptase–PCR with specific sets of primers for *Id3*, *Hes-1,* and glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) (*Right*). (*B*) Neuroepithelial cells were cotransfected with −179H5-Luc and pRL-CMV and a control vehicle or a construct expressing *Smad7* and then stimulated with BMP2 for 8 h. (*C*) Neuroepithelial cells were treated as in *B*, except that −1575Id1-Luc was used instead of −179H5-Luc. Reporter constructs containing various lengths of the *Hes-5* (*D*) or *Id1* (*E*) promoter were cointroduced with pRL-CMV into neuroepithelial cells as indicated, and cells were stimulated with BMP2 for 8 h.

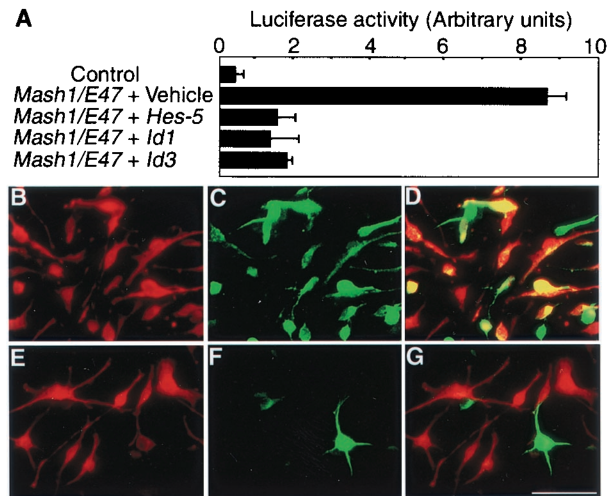

Fig. 5. Effect of negative HLH proteins on the function of Mash1 and neurogenesis in cultured neural precursor cells. (*A*) Neuroepithelial cells were cotransfected with pKE7-βA(core)-luc and pRL-CMV along with the constructs indicated. Luciferase activity was measured 32 h after the transfection. (*B*--*G*) Neuroepithelial cells were infected with adenoviruses that had been engineered to express *LacZ* or FLAG-tagged *Id1*. Two days after the infection, the cells were stained with antibodies against LacZ (*B*) (red) or FLAG (*E*) (red) and an antibody against MAP2 (*C* and *F*) (green). Superimposed views of *B* and *C* are in *D* and of *E* and *F* are in *G*. (Bar, 50 μm.)

expression of Mash1 was down-regulated by BMP2 in the neuroepithelial cells. Reverse transcriptase–PCR analysis indicated that expression of this proneural gene was not significantly inhibited by BMP2 (up to 24 h after stimulation; data not shown). Thus, we focused our attention on nuclear factors that inhibit the transcriptional activity of bHLH proteins. As shown in Fig. 4*A*, expression of the negative HLH genes *Hes-5*, *Id1,* and *Id3*, but not *Hes-1*, was up-regulated by BMP2 stimulation in neuroepithelial cells. In agreement with this result, BMP2 clearly induced promoter activation of *Hes-5* and *Id1* genes (Fig. 4 *B* and *C*). BMP2 activates transcription factors Smad1, -5, or -8; this activity is suppressed by the inhibitory Smad proteins Smad6 and Smad7 (5–7). As shown in Fig. 4 *B* and *C*, Smad7 reduced the BMP2-induced promoter activation of *Hes-5* and *Id1* genes to almost basal levels, suggesting that activation of Smad proteins is required for the induction of *Hes-5* and *Id1* gene expression. We next tried to determine a BMP2-responsive region in the promoter of these genes (32, 33). When the sequences between positions −179 and −141 or between positions −1147 and −927 were deleted in the *Hes-5* or *Id1* promoter, respectively, responsiveness of each promoter to BMP2 was diminished considerably (Fig. 4 *D* and *E*). Each important region contains one or seven motifs, respectively, which is (or are) identical or similar to the putative BMP-responsive Smad-binding motif GCCGNCGC (34–36).

Effect of Negative HLH Proteins on the Function of Neurogenic Transcription Factor Mash1 and Neurogenesis of Neural Precursor Cells. To determine whether Hes-5, Id1, or Id3, whose expression is induced by BMP2, inhibit the transcriptional activity of the Mash1/E47 neurogenic bHLH protein complex on an E-box-containing promoter (20, 21, 37), we cotransfected cells with pKE7-βA(core)-luc and pRL-CMV along with HES-5, Id1, or Id3 expression construct. Expression of any of these negative HLH regulators led to a reduction in promoter activation induced by the Mash1–E47 complex (Fig. 5*A*). Similar inhibition by the negative HLH regulators was observed for the neurogenin–E47 complex (data not shown). Thus, functions of other proneural gene products, such as Math and NeuroD family proteins, could also be inhibited by these negative HLH regulators. It may be notable that HES-5, Id1, and Id3 do not have to be present at the same time to exert the antineurogenic effect of BMP2, because neuronal differentiation was inhibited normally by BMP2 in our culture system using the neuroepithelial cells from *Hes-5*-deficient mice (unpublished data; for more information about *Hes-5*-deficient mice, see ref. 38). It is likely that other negative HLH regulators, including Id1 and Id3, might compensate for loss of the *Hes-5* gene product in the inhibition of neurogenesis by BMP2.

To confirm whether Id1 and Id3 can actually inhibit neurogenesis, neuroepithelial cells were infected with recombinant adenovirus engineered to express β-galactosidase (LacZ), Id1, or Id3. When the percentage of LacZ-expressing cells that also expressed MAP2 after 2-day culture was determined, 39.4% of the cells were MAP2-positive neurons, which is comparable to that observed without infection (see Fig. 1*A*). The percentage of MAP2-positive cells that also expressed virus-vector-derived Id1 or Id3 was markedly reduced to 14.8% and 14.3%, respectively, which is comparable to the frequency of MAP2-positive cells in

a 2-day culture with BMP2 (see Fig. 1*B*). Representative immunofluorescently stained cells are shown in Fig. 5 *B–G*. These results indicate that the expression of Id1 and Id3 is sufficient to inhibit the neurogenic program in neural progenitor cells.

Discussion

We have shown in the present study that transient exposure of neural precursor cells to BMP2 is sufficient to switch the developmental pathway of these cells from neurogenesis to astrocytogenesis. In addition, we have also suggested that this effect is mediated by the Smad-dependent expression of negative HLH proteins such as Id1 and Id3. We show that ectopic expression of these two Id proteins was sufficient to inhibit the neurogenic fate. The expression of Id1 and/or Id3 proteins, however, does not seem to be sufficient to induce astrocytogenesis, because forced expression of these Id proteins in neuroepithelial cells, as shown in Fig. 5, was not able to induce GFAP-positive cells even when cells were cultured with LIF for 4 days (unpublished data). We assume that both Smads and STAT3, which are activated by BMP2 and LIF, respectively, are required for astrocyte differentiation (12, 13, 39), but Smads could not be activated in the adenovirus-mediated Id expression system tested. Furthermore, expression of Id1 and/or Id3 proteins might have to be transient. In this regard, we observed that BMP2-induced expression of these two genes in cultured neuroepithelial cells was transient (reaching a peak level at 1 h and returning to the basal level within 24 h after BMP2 stimulation; unpublished data). In addition, expression of *Id1* and *Id3* is not observed in the brain at a late stage of development when GFAP-positive astrocytes are detected (40–43). It should be noted that retrovirus-mediated expression of Id1 in cerebral cortex of embryonic or postnatal mouse leads to suppression of neurogenesis and induction of gliogenesis, as judged by cellular morphology, although the expression of glial marker proteins, such as GFAP, was not examined (44). It is conceivable that certain external signals in the brain *in vivo* may cooperate with Id1 to promote gliogenesis.

Among the four Id proteins identified to date, expression patterns of *Id1* and *Id3* (in proliferating neural precursors in the ventricular zone) in the developing brain are quite similar (40–43). BMP receptors are also expressed in cells located in the ventricular zone at a developmental stage when *Id* expression is observed (14). Therefore, the mechanism whereby BMP2 inhibits neurogenesis may also operate under physiological condition. In support of this, enhanced neurogenesis has been observed in the brain of *Id1-Id3* double knockout mice (43). Furthermore, overexpression of Id2 protein, which has been shown to impede the function of tissue-specific bHLH proteins, inhibited neuronal differentiation in a similar experimental system (45), although we have not examined whether its expression is regulated by BMP2. This finding further supports the proposed mechanism in which BMP2 exerts its antineurogenic effect via negative HLH regulators. Several proneural bHLH factors have been identified. Besides Mash1 (Fig. 5) and neurogenin (data not shown), we have not identified all of the proneural bHLH targets of Id proteins, but there may be many bHLH targets that are involved in the antineurogenic effect of BMP2.

BMP2 also appears to inhibit neurogenesis in cultured olfactory epithelial cells (46). The authors suggest that the antineurogenic effect of BMP2 is attributed to rapid proteolysis of Mash1 by an unknown mechanism, although they did not test Id proteins.

In contrast to our results obtained with cells from the developing CNS, BMP2 may induce neuronal differentiation in the peripheral nervous system (PNS), as observed with neural crest stem cells (47–49). *Mash1* expression is induced in the neural crest stem cells in response to BMP2, which seems to be responsible for the neurogenic action of BMP2 in PNS (47–49). Our results with CNS precursors may, thus, cast a new light on the molecular mechanisms underlying differential regulation of cell fate in CNS and PNS by BMP2.

Transient Notch activation initiates an irreversible switch from neurogenesis to gliogenesis by neural crest stem cells (50) and adult hippocampus-derived multipotent progenitors (51). The Notch downstream signaling molecules Hes-1 and Hes-5, the latter of which was shown to be induced by BMP2 in the present study, have been suggested to inhibit neurogenesis and induce Müller glial differentiation in the retina (52, 53). Hes-5-deficient retina showed a 30–40% decrease in the number of Müller glial cells (53).

Neural stem cells, endogenously present in spinal cord *in vivo,* proliferate in response to injury, yet the vast majority of newly generated cells are GFAP-positive astrocytes (54). In addition, adult hippocampus-derived neural stem cells, when implanted into adult brain in such a region as cerebellum or striatum, have been reported to differentiate predominantly into glial cells (2, 55, 56). Inhibitors of BMP-signaling such as Noggin, Chordin, and inhibitory Smads may contribute to the promotion of neurogenesis, instead of the naturally occurring gliogenic response, of endogenously present or engrafted neural stem cells. In support of this hypothesis, ectopic expression of the BMP antagonist Noggin in the adult mouse striatum, where BMP2 and BMP4 are normally expressed (11), promoted neuronal differentiation of grafted neural progenitors (16). Thus, strategies that inhibit BMP signaling could be used to treat spinal cord injury and other CNS degenerative diseases.

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